

82

## SEARCH REQUEST FORM

## Scientific and Technical Information Center

Requester's Full Name: RITA MITRA Examiner #: 77995 Date: 3/22/02  
 Art Unit: 1653 Phone Number 301-605-1211 Serial Number: 07/547463  
 Mail Box and Bldg/Room Location: 9801 CMV Results Format Preferred (circle): PAPER DISK E-MAIL  
LM 7803

If more than one search is submitted, please prioritize searches in order of need.

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Recombinant Protein Production in a Human cell

Inventors (please provide full names): Guus Hattemerker, Karina Cornelia Verhulst  
Govert Schouten, Alphonsus Uytdehaag, Abraham Bont

Earliest Priority Filing Date: 4/15/1999

\*For Sequence Searches Only\* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.

I request an expedited literature search (Patent and Non Patent) because this case is due this bi-week. No Sequence Search is required.

Claims 1, 3, 5 - 7, 11, 13, 16, Branch 22 were elected,

Mary Hale Supervisor, Info. Branch  
 STIC - Biotech/Chear. Library  
 CM-1 Room ED1  
 703-308-4208

## Keyword:

- method of producing proteinaceous substance
- adenoviral E1 protein
- eukaryotic mammalian cell
- adenoviral E1A protein
- post-translational modification
- peri-translational modification
- crythropoietin
- E2A protein
- E1B protein

PLEASE RUSH

Days that

This search was done before doing next.  
 Scl. elected 1, 3, 5 - 7, 11, 13, 14, 17, 36, 97

27/37  
 44/41  
 68/65

## STAFF USE ONLY

## Type of Search

## Vendors and cost where applicable

Searcher: 1101

NA Sequence (#)

STN

76 85

Searcher's Name: 1101

Job#

Searcher Location: \_\_\_\_\_

Structure (#)

Questel/Orbit

Date Searcher Picked Up: \_\_\_\_\_

Bibliographic

Dr. Link

Date Completed: 6/3

Litigation

Lexis/Nexis

Searcher Prep & Review Time: \_\_\_\_\_

Fulltext

Sequence Systems

Clerical Prep Time: \_\_\_\_\_

Patent Family

WWW/Internet

Online Time: 5

Other

Other (specify)



UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS  
UNITED STATES PATENT AND TRADEMARK OFFICE  
WASHINGTON, D.C. 20231  
www.uspto.gov



Bib Data Sheet

SERIAL NUMBER 09/549,463	FILING DATE 04/14/2000 RULE	CLASS 514	GROUP ART UNIT 1614	ATTORNEY DOCKET NO. 4038.1US
-----------------------------	-----------------------------------	--------------	------------------------	------------------------------------

**APPLICANTS**

Guus Hatteboer, Heemstede, NETHERLANDS;  
Karina Cornelia Verhulst, Leiden, NETHERLANDS;  
Govert Johan Schouten, Leiderdorp, NETHERLANDS;  
Alphonsus Gerardus Uytdehaag, DeBilt, NETHERLANDS;  
Abraham Bout, Moerkapelle, NETHERLANDS;

**\*\* CONTINUING DATA \*\*\*\*\***

THIS APPLN CLAIMS BENEFIT OF 60/129,452 04/15/1999

**\*\* FOREIGN APPLICATIONS \*\*\*\*\***

IF REQUIRED, FOREIGN FILING LICENSE  
GRANTED \*\* 07/05/2000

\*\* SMALL ENTITY \*\*

Foreign Priority claimed	<input type="checkbox"/> yes <input type="checkbox"/> no	STATE OR COUNTRY NETHERLANDS	SHEETS DRAWING 27	TOTAL CLAIMS 71	INDEPENDENT CLAIMS 7
35 USC 119 (a-d) conditions met	<input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> Met after Allowance				
Verified and Acknowledged	Examiner's Signature	Initials			

**ADDRESS**

Allen C Turner  
Trask Britt & Rossa  
P. O. Box 2550  
Salt Lake City ,UT 84110

**TITLE**

Recombinant protein production in a human cell

FILING FEE RECEIVED 2237	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	<input type="checkbox"/> All Fees
		<input type="checkbox"/> 1.16 Fees ( Filing )
		<input type="checkbox"/> 1.17 Fees ( Processing Ext. of time )
		<input type="checkbox"/> 1.18 Fees ( Issue )
		<input type="checkbox"/> Other _____
		<input type="checkbox"/> Credit



RECEIVED

1653

JAN 28 2002

TECH CENTER 1600/2900

PATENT

*307  
JW*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Hateboer et al.

Serial No.: 09/549,463

Filed: May 14, 2000

For: RECOMBINANT PROTEIN  
PRODUCTION IN A HUMAN CELL

Examiner: R. Mitra

Group Art Unit: 1653

Attorney Docket No.: 4038.1US

CERTIFICATE OF MAILING

I hereby certify that this correspondence along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service as First Class Mail (under 37 C.F.R. § 1.8(a)) on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner for Patents, Washington, D.C. 20231.

November 2, 2001  
Date of Deposit

*Lynette Ellison* 2/16/02  
Signature of registered practitioner or other person having reasonable basis to expect mailing to occur on date of deposit shown pursuant to 37 C.F.R. § 1.8(a)(1)(ii).

*Lynette Ellison*  
Typed/printed name of person whose signature is contained above

AMENDMENT

Box Non-Fee Amendment  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

Responsive to the Office Action mailed October 2, 2001, Applicants elect the invention of Group I (i.e., claims 1, 3, 5-7, 11, 13, 14 and 22) without traverse. This election is made without prejudice to pursue the remaining claims in a related application.

DISK TO STIC

DATE: \_\_\_\_\_



**IN THE SPECIFICATION:**

Pursuant to 37 C.F.R. §§ 1.121 and 1.125 (as amended to date) please enter the substitute specification in clean form and including paragraph numbers [0001] through [0196], references, tables and Abstract attached hereto as Appendix A. A marked-up substitute specification to clearly identify amendments to the specification as required by 37 C.F.R. § 1.121(b)(3)(iii) is attached hereto as Appendix B. It is respectfully submitted that the substitute specification does not introduce new matter into the above-referenced patent application.

Please add the attached sequence listing to the above referenced application after the claims.

**IN THE CLAIMS:**

Please cancel claims 2, 4, 8, 9, 18-21, 23-34, 37-41, 43, 45, 47, 49, 51-54, 57, 58, 63, 64, and 69-72 without prejudice or disclaimer.

B1 ✓ 5. (Twice amended) The method according to claim 1, wherein at least one of the proteinaceous substance harvested is encoded by said gene.

B2 ✓ 7. (Twice amended) The method according to claim 1, wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

B3 ✓ 11. (Twice amended) The method according to claim 1, wherein said proteinaceous substance is a protein that undergoes post-translational and/or peri-translational modification.

*processing* *genetic translation*  
*protein processing (a) post translational*  
*post translational protein modification*

B4 ✓ 13. (Twice amended) The method according to claim 1, wherein said proteinaceous substance is erythropoietin.

*anemia disease*  
*glycoprotein hormone*  
*receptor (a) erythropoietin*  
*adenovirus E1A protein*

*transcription factors*  
*adenovirus early protein*  
*E1A protein*  
*antigen (a) viral (a) tumor*  
*oncogene protein (a) viral*  
*tumour*

✓ 22. (Amended) A recombinant mammalian cell immortalized by the presence of at least one adenoviral E1A protein or a functional derivative, homologue and/or fragment thereof, said recombinant mammalian cell comprising:

*BS*  
a nucleic acid in a functional format for expressing at least one variable domain of an immunoglobulin or a functional derivative, homologue and/or fragment thereof; and a nucleic acid derived from an adenovirus encoding said at least one E1A protein.

Please add the following new claims:

73. The method according to claim 6, wherein said human recombinant protein is a protein that undergoes post-translational and/or peri-translational modification.

74. The method according to claim 6, wherein said human recombinant protein is erythropoietin.

75. The method according to claim 74, wherein said eukaryotic cell produces in excess of 100 units erythropoietin thereof per million cells in 24 hours.

76. The method according to claim 1, wherein said eukaryotic cell is a human cell.

*P*  
77. The method according to claim 1, wherein said proteinaceous substance comprises a viral protein other than an adenoviral protein.

78. The method according to claim 3, wherein said proteinaceous substance comprises a viral protein other than an adenoviral protein.

✓ 79. The method according to claim 11, wherein said proteinaceous substance comprises a viral protein other than an adenoviral protein.

80. The method according to claim 6, wherein said human recombinant protein comprises a viral protein other than an adenoviral protein.

81. The method according to claim 7, wherein said human recombinant protein comprises a viral protein other than an adenoviral protein.

82. The method according to claim 77, where said viral protein is selected from the group consisting of: an influenza virus neuramidase and/or a hemagglutinin; an enterovirus protein or a functional equivalent thereof; a herpes virus protein or a functional equivalent thereof; an orthomyxovirus protein; a retrovirus, a parvovirus or a popavovirus protein; a rotavirus or a coronavirus protein; a togavirus protein, rubella virus protein or an Eastern-, Western-, or Venezuelan equine encephalomyelitis virus protein; a hepatitis causing virus protein, a hepatitis A protein, or a hepatitis B virus protein; and a pestivirus protein, such as hog cholera virus protein or a rhabdovirus protein, such as a rabies virus protein.

83. The method according to claim 78, where said viral protein is selected from the group consisting of: an influenza virus neuramidase and/or a hemagglutinin; an enterovirus protein or a functional equivalent thereof; a herpes virus protein or a functional equivalent thereof; an orthomyxovirus protein; a retrovirus, a parvovirus or a popavovirus protein; a rotavirus or a coronavirus protein; a togavirus protein, rubella virus protein or an Eastern-, Western-, or Venezuelan equine encephalomyelitis virus protein; a hepatitis causing virus protein, a hepatitis A protein, or a hepatitis B virus protein; and a pestivirus protein, such as hog cholera virus protein or a rhabdovirus protein, such as a rabies virus protein.

84. The method according to claim 79, where said viral protein is selected from the group consisting of: an influenza virus neuramidase and/or a hemagglutinin; an enterovirus protein or a functional equivalent thereof; a herpes virus protein or a functional equivalent thereof; an orthomyxovirus

protein; a retrovirus, a parvovirus or a popavovirus protein; a rotavirus or a coronavirus protein; a togavirus protein, rubella virus protein or an Eastern-, Western-, or Venezuelan equine encephalomyelitis virus protein; a hepatitis causing virus protein, a hepatitis A protein, or a hepatitis B virus protein; and a pestivirus protein, such as hog cholera virus protein or a rhabdovirus protein, such as a rabies virus protein.

✓ 85. The method according to claim 80, where said viral protein is selected from the group consisting of: an influenza virus neuramidase and/or a hemagglutinin; an enterovirus protein or a functional equivalent thereof; a herpes virus protein or a functional equivalent thereof; an orthomyxovirus protein; a retrovirus, a parvovirus or a popavovirus protein; a rotavirus or a coronavirus protein; a togavirus protein, rubella virus protein or an Eastern-, Western-, or Venezuelan equine encephalomyelitis virus protein; a hepatitis causing virus protein, a hepatitis A protein, or a hepatitis B virus protein; and a pestivirus protein, such as hog cholera virus protein or a rhabdovirus protein,

*cont* b such as a rabies virus protein.

86. The method according to claim 81, where said viral protein is selected from the group consisting of: an influenza virus neuramidase and/or a hemagglutinin; an enterovirus protein or a functional equivalent thereof; a herpes virus protein or a functional equivalent thereof; an orthomyxovirus protein; a retrovirus, a parvovirus or a popavovirus protein; a rotavirus or a coronavirus protein; a togavirus protein, rubella virus protein or an Eastern-, Western-, or Venezuelan equine encephalomyelitis virus protein; a hepatitis causing virus protein, a hepatitis A protein, or a hepatitis B virus protein; and a pestivirus protein, such as hog cholera virus protein or a rhabdovirus protein, such as a rabies virus protein.

88. The method according to claim 1, wherein said eukaryotic cell further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in its genome.

89. The method according to claim 6, wherein said eukaryotic cell further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in its genome.

90. The method according to claim 88, wherein said E2A encoding sequence encodes a temperature sensitive mutant E2A.

91. The method according to claim 89, wherein said E2A encoding sequence encodes a temperature sensitive mutant E2A.

*conclude*  
92. A recombinant erythropoietin molecule produced by the method of claim 1.

*3* b  
93. A recombinant erythropoietin molecule produced by the method of claim 6.

94. The recombinant protein of claim 92 wherein said recombinant protein has a human glycosylation pattern different from that of the protein's isolated natural counterpart protein.

95. The recombinant protein of claim 93 wherein said recombinant protein has a human glycosylation pattern different from that of the protein's isolated natural counterpart protein.

96. The recombinant mammalian cell of claim 22, further comprising:  
a nucleic acid derived from an adenovirus encoding an E1B protein.

97. The method according to claim 6, wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

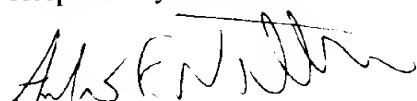
added claims 73-92 such that the claims are directed to the subject matter of Group I. Substantive examination of the application is requested.

A substitute specification without claims and sequence listing are also being provided. It is respectfully submitted that no new matter has been added in the substitute specification or sequence listing.

### CONCLUSION

If questions exist after consideration of the foregoing, the Office is kindly requested to contact the applicants' representative at the address or telephone number below.

Respectfully submitted,



Andrew F. Nilles  
Registration No. 47,825  
Attorney for Applicants  
TRASKBRITT, PC  
P. O. Box 2550  
Salt Lake City, Utah 84110-2550  
Telephone: (801) 532-1922

AFN

Date: November 2, 2001

Attachments:      Marked up Version of Claims  
                         Paper copy of Sequence Listing  
                         Appendix A: Clean Version of Substitute Specification  
                         Appendix B: Marked Up Version of Substitute Specification  
                         Statement under 37 C.F.R. §§ 1.821 through 1.825  
                         Computer Readable Form of Sequence Listing

N:\2183\4038.1\Response to restriction requirement.wpd

### MARKED UP VERSION OF CLAIMS

**MARKED UP VERSION OF CLAIMS**

5. (Twice amended) The method according to claim 1 [or claim 2], wherein at least one of the [at least one] proteinaceous substance harvested is encoded by said gene.

7. (Twice amended) The method according to [any one of] claim[s] 1[, 2 or 6], wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

11. (Twice amended) The method according to [any one of] claim[s] 1[, 2 or 6], wherein said proteinaceous substance is a protein that undergoes post-translational and/or peri-translational modification[s].

13. (Twice amended) The method according to [any one of] claim[s] 1,[ 2 or 6,] wherein said proteinaceous substance is erythropoietin[, or a functional derivative, homologue or fragment thereof].

22. (Amended) A recombinant mammalian cell immortalized by the presence of at least one adenoviral E1A protein or a functional derivative, homologue and/or fragment thereof, said recombinant mammalian cell comprising:

a nucleic acid in a functional format for expressing at least one variable domain of an immunoglobulin or a functional derivative, homologue and/or fragment thereof; and  
a [The recombinant mammalian cell of claim 21, wherein said] nucleic acid derived from an adenovirus encod[es]ing said at least one [an] E1A [and/or an E1B] protein.

45/A  
12-23-00  
M.L.

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:**

Hateboer et al.

**Serial No.:** To be assigned

**Filed:** April 14, 2000

**For:** RECOMBINANT PROTEIN  
PRODUCTION IN A HUMAN CELL

**Examiner:** To be assigned

**Group Art Unit:** To be assigned

**Attorney Docket No.:** 4038.1US

**NOTICE OF EXPRESS MAILING**

Express Mail Mailing Label Number: EL500246665US

Date of Deposit with USPS: April 14, 2000

Person making Deposit: Jared Turner

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please ~~revise~~ the above-identified application as follows:

**IN THE CLAIMS:**

Please ~~cancel~~ claims 10, 12 without prejudice or disclosure. Please ~~cancel~~ claims 15,  
17, 35, 36, 42, 44, 46, 48, 50, 55, 56, 59-62 and 65-68 without prejudice or disclaimer.

Please amend the claims as follows:

3. (Amended) The method according to claim 1 [or claim 2], wherein said eukaryotic cell is a  
mammalian cell.

A1

*A2* *B1* 5. (Amended) The method according to [any one of claims 1 to 4] claim 1 or claim 2, wherein at least one of the at least one proteinaceous substance harvested is encoded by said gene.

*A2* *B2* 7. (Amended) The method according to any one of [the foregoing claims] claims 1, 2 or 6, wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

*A3* *B3* 8. (Amended) The method according to any one of [the foregoing claims] claims 1, 2 or 6, wherein said at least one adenoviral E1 protein comprises an E1B protein or a functional homologue, fragment and/or derivative thereof.

*B* 9. (Amended) The method according to [any one of claims 1 through 8] claim 8, wherein said eukaryotic cell produces from about 2 to about 200-fold more recombinant protein and/or proteinaceous substance than conventional mammalian cell lines.

*A4* *B3* 11. (Amended) The method according to any one of claims 1 [to 10], 2 or 6, wherein said proteinaceous substance is a protein that undergoes post-translational and/or peri-translational modifications.

*A5* *B4* 13. (Amended) The method according to any one of claims [1-12] 1, 2 or 6, wherein said proteinaceous substance is erythropoietin, or a functional derivative, homologue or fragment thereof.

*A4* *Cm* 20. (Amended) The recombinant mammalian cell of claim 18 [or claim 19], wherein said at least one adenoviral *E1* protein comprises an E1B protein or a functional homologue, fragment and/or derivative thereof.

*A6*  
*cmcl*  
21. (Amended) The recombinant mammalian cell of [any one of claims 18 to 20]claim 18, comprising a nucleic acid derived from an adenovirus encoding said at least one adenoviral E1 protein.

*A7*  
23. (Amended) The recombinant mammalian cell of [any one of claims 18-22]claim 18, wherein said recombinant mammalian cell is derived from a primary cell.

24. (Amended) The recombinant mammalian cell of [any one of claims 18 through 23]claim 18, which recombinant mammalian cell is derived from a human cell.

*A8*  
26. (Amended) The recombinant mammalian cell of [any one of claims 18 through 25]claim 25, wherein said cell further comprises a nucleic acid encoding E2A or a functional homologue, fragment and/or derivative thereof.

*A9*  
28. (Amended) The recombinant mammalian cell of [any one of claims 18 through 27]claim 18, wherein said nucleic acid in a functional format for expressing at least one variable domain, encodes a heavy chain, a variable heavy chain, a light chain and/or a variable light chain of an immunoglobulin.

*cm†*  
29. (Amended) The recombinant mammalian cell of [any one of claims 18 through 28]claim 18, further comprising another nucleic acid in functional format for expressing at least one counterpart of said at least one variable domain.

30. (Amended) The recombinant mammalian cell of [any one of claims 18 through 29]claim 18, wherein said nucleic acid in functional format for expressing at least one variable domain and/or at least one counterpart thereof encodes an ScFv.

31. (Amended) The recombinant mammalian cell of [any one of claims 18 through 30]claim 18, wherein at least one of said variable domains comprises a human or humanized amino acid sequence.

32. (Amended) The recombinant mammalian cell of [any one of claim 18 through 31]claim 18, wherein at least one of said variable domains is encoded by a nucleic acid under the control of an inducible promoter.

A9  
cmcl. 33. (Amended) A process for producing at least one variable domain of an immunoglobulin, said process comprising:

culturing a recombinant mammalian cell [of any one of claims 18-32, immortalized by the presence of at least one adenoviral E1 protein or a functional derivative, homologue and/or fragment thereof, said recombinant mammalian cell comprising a nucleic acid in a functional format for expressing at least one variable domain of an immunoglobulin or a functional derivative, homologue and/or fragment thereof in a suitable medium; and

harvesting said at least one variable domain of an immunoglobulin from said recombinant mammalian cell and/or said medium.

A10 37. (Amended) A variable domain of an immunoglobulin, or a functional part, homologue or derivative thereof, produced by the process of [any one of claims]claim 33 [through 36].

A11 57. (Amended) The method according to any one of claims [1-17, 33, 34, 39-53, 55 or 56]1, 2 or 6, wherein said eukaryotic cell further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in its genome.

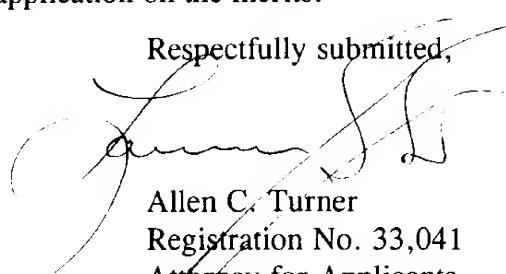
A12  
63. (Amended) A recombinant erythropoietin molecule [obtainable by a method according to any one of claims 1-17 and 55-62] produced by the method of any one of claims 1, 2 or 6.

A13  
72. (Amended) The recombinant mammalian cell of [any one of] claim 18 [through 31], wherein an endogenous DHFR nucleic acid is at least functionally deleted.

REMARKS

No new matter has been added. The Applicants request entry of the foregoing amendment prior to examination of the application on the merits.

Respectfully submitted,

  
Allen C. Turner  
Registration No. 33,041  
Attorney for Applicants  
TRASK, BRITT & ROSSA  
P. O. Box 2550  
Salt Lake City, Utah 84110-2550  
Telephone: (801) 532-1922

rg # 30,549

Date: April 14, 2000

ACT/bv

N:\2183\4038.1\PRE.AMD

## Claims

✓ 1. A method for producing at least one proteinaceous substance in a eukaryotic cell, said method comprising:

5 providing a eukaryotic cell having a nucleic acid sequence in the eukaryotic cell's genome, said nucleic acid sequence encoding at least one adenoviral E1 protein or a functional homologue, fragment or derivative thereof, which eukaryotic cell further does not encode a structural adenoviral protein in its genome or a sequence integrated therein;

10 providing said eukaryotic cell with a gene encoding a recombinant proteinaceous substance;

culturing said eukaryotic cell in a suitable medium; and  
harvesting at least one proteinaceous substance from said eukaryotic cell, said suitable medium, or both said eukaryotic cell and said medium.

15 2. A method for enhancing production of a recombinant proteinaceous substance in a eukaryotic cell, said method comprising:

20 providing said eukaryotic cell with a gene encoding at least part of a proteinaceous substance, wherein said nucleic acid is under control of a CMV-promoter, an E1A promoter, or a functional homologue, derivative and/or fragment of either; and

providing said eukaryotic cell with adenoviral E1A-activity or E1A-like activity.

25 3. The method according to claim 1 or claim 2, wherein said eukaryotic cell is a mammalian cell.

4. The method according to claim 3, wherein said eukaryotic cell is a human cell.

*Sub A2* 7 5. The method according to any one of claims 1 to 4, wherein at least one of the at least one proteinaceous substance harvested is encoded by said gene.

6. A method for producing at least one human recombinant protein in a cell, said method  
5 comprising:

10 providing a eukaryotic cell which is human, with a gene encoding a human recombinant protein, having a sequence encoding at least one adenoviral E1 protein or a functional derivative, homologue or fragment thereof in the human cell's genome which human cell further does not produce structural adenoviral proteins;

culturing said human cell in a suitable medium; and

15 harvesting the human recombinant protein from the human cell, the suitable medium, or both said human cell and said medium.

*Sub A3* 15 7. The method according to any one of the foregoing claims, wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

20 8. The method according to any one of the foregoing claims, wherein said at least one adenoviral E1 protein comprises an E1B protein or a functional homologue, fragment and/or derivative thereof.

25 9. The method according to any one of claims 1 through 8, wherein said eukaryotic cell produces from about 2 to about 200-fold more recombinant protein and/or proteinaceous substance than conventional mammalian cell lines.

10. The method according to claim 9, wherein said conventional mammalian cell lines are selected from the group consisting of CHO, COS, Vero, Hela, BHK and Sp-2 cell lines.

*Sub A4* 7 11. The method according to any one of claims 1 to 10 wherein said proteinaceous substance is a protein that undergoes post-translational and/or peri-translational modifications.

5

12. The method according to claim 11, wherein said post-translational and/or peri-translational modifications comprise glycosylation.

*Sub A5* 10 13. The method according to any one of claims 1-12, wherein said proteinaceous substance is erythropoietin, or a functional derivative, homologue or fragment thereof.

14. The method according to claim 13, wherein said eukaryotic cell produces in excess of 100 units erythropoietin thereof per million cells in 24 hours.

15 15. The method according to claim 14, wherein said eukaryotic cell produces in excess of 500 units erythropoietin thereof per million cells in 24 hours.

16. The method according to claim 15, wherein said eukaryotic cell produces in excess of 1000 units erythropoietin thereof per million cells in 24 hours.

20

17. The method according to claim 16, wherein said eukaryotic cell produces in excess of 5000 units erythropoietin or functional derivatives thereof per million cells in 24 hours.

25

18. A recombinant mammalian cell immortalized by the presence of at least one adenoviral E1 protein or a functional derivative, homologue and/or fragment thereof, said recombinant mammalian cell comprising a nucleic acid in a functional format for expressing at least one variable domain of an immunoglobulin or a functional derivative, homologue and/or fragment thereof.

*Sub A6* 5  
19. The recombinant mammalian cell of claim 18, wherein said at least one adenoviral E1 protein comprises an E1A protein or a functional homologue, fragment and/or derivative thereof.

10  
20. The recombinant mammalian cell of claim 18 or claim 19, wherein said at least one adenoviral E1 protein comprises an E1B protein or a functional homologue, fragment and/or derivative thereof.

15  
21. The recombinant mammalian cell of any one of claims 18 to 20, comprising a nucleic acid derived from an adenovirus encoding said at least one adenoviral E1 protein.

*Sub B* 22. The recombinant mammalian cell of claim 21, wherein said nucleic acid derived from an adenovirus encodes an E1A and/or an E1B protein.

25  
23. The recombinant mammalian cell of any one of claims 18-22, wherein said recombinant mammalian cell is derived from a primary cell.

20  
24. The recombinant mammalian cell of any one of claims 18 through 23, which recombinant mammalian cell is derived from a human cell.

25  
25. The recombinant mammalian cell according to claim 24, deposited as ECACC no. 96022940 or a derivative thereof.

*Sub A8* 26. The recombinant mammalian cell of any one of claims 18 through 25, wherein said cell further comprises a nucleic acid encoding E2A or a functional homologue, fragment and/or derivative thereof.

27. The recombinant mammalian cell of claim 26, wherein said nucleic acid encoding E2A comprises a temperature sensitive mutant E2A.

1 28. The recombinant mammalian cell of any one of claims 18 through 27, wherein said  
nucleic acid in a functional format for expressing at least one variable domain, encodes a  
heavy chain, a variable heavy chain, a light chain and/or a variable light chain of an  
immunoglobulin.

5 29. The recombinant mammalian cell of any one of claims 18 through 28, further  
comprising another nucleic acid in functional format for expressing at least one  
counterpart of said at least one variable domain.

10 30. The recombinant mammalian cell of any one of claims 18 through 29, wherein said  
nucleic acid in functional format for expressing at least one variable domain and/or at  
least one counterpart thereof encodes an ScFv.

15 31. The recombinant mammalian cell of any one of claims 18 through 30, wherein at least  
one of said variable domains comprises a human or humanized amino acid sequence.

20 32. The recombinant mammalian cell of any one of claim 18 through 31, wherein at least  
one of said variable domains is encoded by a nucleic acid under the control of an  
inducible promoter.

25 33. A process for producing at least one variable domain of an immunoglobulin, said  
process comprising:

                  culturing a recombinant mammalian cell of any one of claims 18-32, in a  
                  suitable medium; and

                  harvesting said at least one variable domain of an immunoglobulin from  
                  said recombinant mammalian cell and/or said medium.

34. The process according to claim 33, wherein said recombinant mammalian cell is capable of producing in excess of 10 grams of said at least one variable domain of an immunoglobulin per 10<sup>6</sup> cells per day.

5 35. A process for producing at least one variable domain of an immunoglobulin having post-translational modifications different than that of the variable domain of an immunoglobulin's isolated natural counterparts, said process comprising:

transforming the recombinant mammalian cell of any one of claims 18 through 32 with a gene coding for the variable domain of the immunoglobulin;  
10 culturing the recombinant mammalian cell in a suitable medium; and  
harvesting the at least one variable domain of an immunoglobulin from  
said recombinant mammalian cell and/or said suitable medium.

15 36. The process according to claim 35, wherein said recombinant mammalian cell produces said at least one variable domain of an immunoglobulin, in excess of 10 g per 10<sup>6</sup> cells per day.

20 37. A variable domain of an immunoglobulin, or a functional part, homologue or derivative thereof, produced by the process of any one of claims 33 through 36.

38. The variable domain of an immunoglobulin according to claim 37 together with a suitable carrier forming a pharmaceutical composition.

25 39. The method according to any one of claims 1-12, wherein said proteinaceous substance comprises a viral protein other than an adenoviral protein.

40. The method according to claim 39, wherein said viral protein comprises an influenza virus neuramidase and/or a hemagglutinin.

41. The method according to claim 39, wherein said viral protein comprises an enterovirus protein or a functional equivalent thereof

5 42. The method according to claim 41, wherein said enterovirus protein is selected from the group consisting of rhinovirus, aphto virus, and poliomyelitis virus protein.

43. The method according to claim 39, wherein said viral protein comprises a herpes virus protein or a functional equivalent thereof.

10 44. The method according to claim 43, wherein said herpes virus protein comprises a protein selected from the group consisting of herpes simplex virus, pseudorabies virus and bovine herpes virus protein.

15 45. The method according to claim 39, wherein said virus protein comprises an orthomyxovirus protein.

46. The method according to claim 45, wherein said orthomyxovirus protein is selected from the group consisting of an influenza virus, a paramyxovirus, such as Newcastle Disease virus, a respiratory syncitio virus, a mumps virus and a measles virus protein.

20 47. The method according to claim 39, wherein said virus protein comprises a retrovirus, a parvovirus or a popovavirus protein.

25 48. The method according to claim 47, wherein said retrovirus protein comprises a human immunodeficiency virus protein.

49. The method according to claim 39, wherein said virus protein comprises a rotavirus or a coronavirus protein.

50. The method according to claim 49, wherein said rotavirus or coronavirus protein is selected from the group consisting of a ~~transmissible~~ gastroenteritisvirus or a flavivirus, such as tick-borne encephalitis virus and yellow fever virus protein.

5 51. The method according to claim 39, wherein said virus protein comprises a togavirus protein, rubella virus protein or an Eastern-, Western-, or Venezuelan equine encephalomyelitis virus protein.

10 52. The method according to claim 39, wherein said virus protein comprises a hepatitis causing virus protein, a hepatitis A protein, or a hepatitis B virus protein.

15 53. The method according to claim 39, wherein said virus protein comprises a pestivirus protein, such as hog cholera virus protein or a rhabdovirus protein, such as a rabies virus protein.

20 54. A process for producing a vaccine comprising a viral protein, said process comprising:

producing the viral protein in a human cell having a sequence encoding at least one adenoviral E1 protein or a functional derivative, homologue or fragment thereof in the human cell's genome, which human cell does not produce a structural adenoviral protein;

harvesting the viral protein;

incorporating the thus harvested viral protein in a vaccine.

25 55. The method according to any one of claims 1-17, 33, 34, or 39-53, wherein said eukaryotic cell is derived from a primary cell.

56. The method according to any one of claims 1-17, 33, 34, 39-53 or 55, wherein said eukaryotic cell is immortalized by the presence of said E1 encoding sequence.

57. The method according to any one of claims 1-17, 33, 34, 39-53, 55 or 56, wherein said eukaryotic cell further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in its genome.

5 58. The method according to claim 57, wherein said E2A encoding sequence encodes a temperature sensitive mutant E2A.

10 59. The method according to any of claims any one of claims 1-17, 33, 34, 39-53, 55-58, wherein said human cell comprises no other adenoviral sequences.

60. The method according to any one of claims 1-17, 33, 34, 39-53, 55-59, wherein said human cell grows in suspension.

15 61. The method according to any one of claims 1-17, 33, 34, 39-53, 55-60, wherein said eukaryotic cell is the PER.C6 cell as deposited under ECACC no. 96022940 or a derivative thereof.

20 62. The method according to any one of claims 1-17, 33, 34, 39-53, 55-61, wherein said human cell is cultured in the absence of serum.

Sub A12 63. A recombinant erythropoietin molecule obtainable by a method according to any one of claims 1-17 and 55-62.

25 64. The recombinant protein claim 63 wherein said recombinant protein has a human glycosylation pattern different from that of the protein's isolated natural counterpart protein.

65. A human cell having a sequence encoding at least one E1 protein of an adenovirus or a functional derivative, homologue or fragment thereof in its genome, which cell does not produce structural adenoviral proteins and having a gene encoding a recombinant protein.

5 66. The human cell of claim 65 which is derived from PER.C6 as deposited under ECACC no. 96022940

67. The human cell of claim 65 or 66, which further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in its genome.

10

68. The human cell of claim 67, wherein said E2A is temperature sensitive.

69. A method of enhancing the production of a proteinaceous substance in a eukaryotic cell an adenoviral E1B protein or a functional derivative, homologue and/or fragment thereof having anti-apoptotic activity, said method comprising providing said eukaryotic cell with an adenoviral E1B protein, derivative, homologue and/or fragment thereof.

15

70. The process according to claim 54, wherein said human cell further comprises a sequence encoding E2A or a functional derivative or analogue or fragment thereof in the human cell's genome.

20

71. The process according to claim 70, wherein said E2A encoding sequence encodes a temperature sensitive mutant E2A.

72. The recombinant mammalian cell of any one of claim 18 through 31, wherein an endogenous DHFR nucleic acid is at least functionally deleted.

Sub A13<sup>25</sup>

add  
B6